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Phytoplankton biomass production in natural lagooning treatment station in Morocco

Production de la biomasse phytoplanctonique dans une station de traitement par lagunage naturel au Maroc

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Abstract. The construction of a station for pretreating wastewater at Aïn Chiffa in the Sefrou city, aims to improve sanitary conditions and to reinforce the protection of water resources. The technique used in this station is «optional lagooning», which is a natural technique of wastewater treatment based on the diseutrophication. This technique is inspired by natural systems of water purification and filtration by microorganisms, algae and aquatic plants. The present study aims to perform an analysis of phytolanctonic biomass in this station during the period January-June 2016. This analysis involves i) Identification of phytoplanktonic species, ii) Enumeration of these species, iii) Determination of the chlorophyll (a) concentration and iv) determination of proteins, carbohydrates and lipids concentrations. Results obtained show spatial and temporal variations in phytoplankton biomasse. The phytoplankton groups detected are Chlorophyceae, Euglenophyceae, Cyanophyceae and Bacillariophyceae. *Chlorella sp*. is present throughout the study period. The study of cellular constituents of phytoplankton shows high concentrations of carbohydrates.

Keywords: Phytoplankton, Natural lagooning, Aïn Chiffa, Sefrou, Morocco.

Résumé. La construction d'une station de pré-traitement des eaux usées à Aïn Chiffa dans la ville de Sefrou vise à améliorer les conditions sanitaires et à renforcer la protection des ressources en eau. La technique utilisée dans cette station est «lagunage facultatif», qu'est une technique naturelle de traitement des eaux usées basée sur la dés-eutrophisation. Cette technique est inspirée des systèmes naturels de purification et de filtration de l'eau par les microorganismes, les algues et les plantes aquatiques. La présente étude vise à effectuer une analyse de la biomasse phytolanctonique dans cette station durant la période Janvier-Juin 2016. Cette analyse passe par i) l'identification des éspéces phytoplanctonique, ii) le dénombrement de ces espèces, iii) la détermination de la concentration en chlorophylle (a) et iv) la détermination de la concentration des protèines, des carbohydrates et des lipides. Les résultats obtenus montrent des variations spatiales et temporelles dans la biomasse phytoplanctonique. Les groupes phytoplanctoniques détectés sont les Chlorophyceae, les Euglenophyceae, les Cyanophyceae et les Bacillariophyceae. *Chlorella sp*. est présente tout au long de la période d'étude. L'étude des constituants cellulaires du phytoplancton montre des concentrations élevées en glucides.

Mots Clés : Phytoplancton, Lagunage naturel, Aïn Chiffa, Séfrou, Maroc.

INTRODUCTION

Water quality is a factor influencing health and mortality in both humans and animals (Kazi *et al*. 2009). Morocco's water resources are limited due to climate conditions, ranging from humid in the north to desertic in the south and episodic droughts. This problematic is not limited to the quantity of water resources, but also to their quality, which must more than ever, well managed (El Addouli *et al.* 2009, Lamrani & Chahlaoui 2012). Wastewater treatment has become a priority today; both to protect the environment and to produce water that can be used in agriculture, industry and other social activities.

Numerous wastewater treatment systems have been developed, including the natural lagooning which is characterized by its operation simplicity, reliability and low investment cost (Oudra 1990). According to Gloyna (1972), lagoon is an ecosystem composed of abiotic substances, productive microorganisms, consumer microorganisms and decomposing microorganisms. Producing microorganisms are represented by algae that collect solar energy to carry out photosynthesis. Algae extract nutrients from the water (nitrogen and phosphorus in particular) and enrich the aquatic environment with oxygen. Decomposing microorganisms (fungi, yeasts and especially bacteria) use this oxygen for organic matter oxidation and produced H_2O, and CO_2 used by algae (Bouarab 2000 and Dekayir 2007). Consuming microorganisms (zooplankton) act as a predator of phytoplankton and bacteria which leads to the reduction in the number of phytoplankton considered as a particular pollution to be eliminated. Zooplankton also constitutes an effective biological filter for elimination autotrophic organic matter (Yang *et al*. 1980, Kawai *et al*. 1987, Kankaala 1988). The micr-oalgae (phytoplankton) characterized by the presence of chlorophyll pigments (Bouarab 2000), need water, oxygen and mineral salts (Bourrelly 1972a). For them, phytoplankton biomass autotrophic can fix solar energy by photosynthesis to convert the mineral carbon into organic matter transferable of the energy to aquatic trophic chain.

In any wastewater treatment station it is necessary to carry out analysis of the raw water and the treated water in order to determine the performance of the treatment used system. The present work aims to (i) study the phytoplankton developed to demonstrate their biodiversity and to (ii) quantify their biochemical composition.

MATERIALS AND METHODS

Study site and sampling

The study area « Aïn Chiffa center » is located in the north (8 km) of Imouzer Kandar city and south (20 km) of Fez city.

Treatment station by natural lagooning is located at 5°01'W and 33°46'N at altitude of 950 m. The weather is typically mediterranean, semi-arid, with an average rainfall of 465 mm/year and annual temperature of 13.5 °C (Fig. 1).

The water samples were collected during the period of January-June 2016 from the four locations in treatment station: (i) the entrance, (ii) the surface of basin (iii) the bottom of basin and (iv) the exit. The water samples were collected in glass bottles and were preserved in ice-boxes and were treated within 12h. The lugol (fixator) was added to the water samples for phytoplankton enumeration. Samples used for chlorophyll (a) determination were stored in the dark.

Phytoplankton analysis

Qualitative analysis (identification by optical microscope)

Phytoplankton identification was carried out by microscopic observation of remarkable morphometric characters of the cells. Identity cells was determined by Bourrelly keys (1972b, 1981, 1985).

Quatitative analysis

Revere microscopy enumeration

At the field, the lugol (fixator) was added to the water samples. At the laboratory, this samples are homogenized and poured into sedimentation flasks. Cells phytoplankton enumeration was carried out according to the Uthermöhl method (NF EN 15204).

Spectrophotometric chlorophyll (a) determination

Chlorophyll (a) concentration per unit of water volume was determined according to the method described by Plante-Cuny (1974). A volume sample (100 ml) was filtered with a Whatman GF/C filter (Ø = 0.45 µm). After they, filter was ground in acetone (90%) and was stored in the dark for 12 h. The filtrate was centrifugated (4000 g/5 min) and the optical density of the supernatant was measured by spectrophotometer at wavelengths 630 nm, 645 nm and 665 nm. Chlorophyll (a) concentration (µg/l) was determined by the formula :

$$\text{Chlorophyl l (a)} = \left(\frac{11.64\ E1 - 2.61\ E2 - 0.1\ E3)\ X\ V}{L\ X\ Vg}\right)$$

E1. Wavelength 665 nm; **E2**. Wavelength 645 nm; **E3**. Wavelength 630 nm; **V**. Volume of acetone (ml); **L**. Diameter of the cuvettes (cm); **Vg**. Volume of filtered water (l).

Biomass study

Samples were pre-filtered (Ø = 160 µm) in order to eliminate zooplankton species and cell debris. After that, filtered samples with a Whatman GF/C filter. The phytoplankton carbohydrates concentration was carried out according to the method described by Dubois *et al*. (1956). Proteins concentration was quantified by method described by Lowry (1951). Lipids were extracted by the chloroform/methanol and quantified by method (Folch *et al*. 1957).

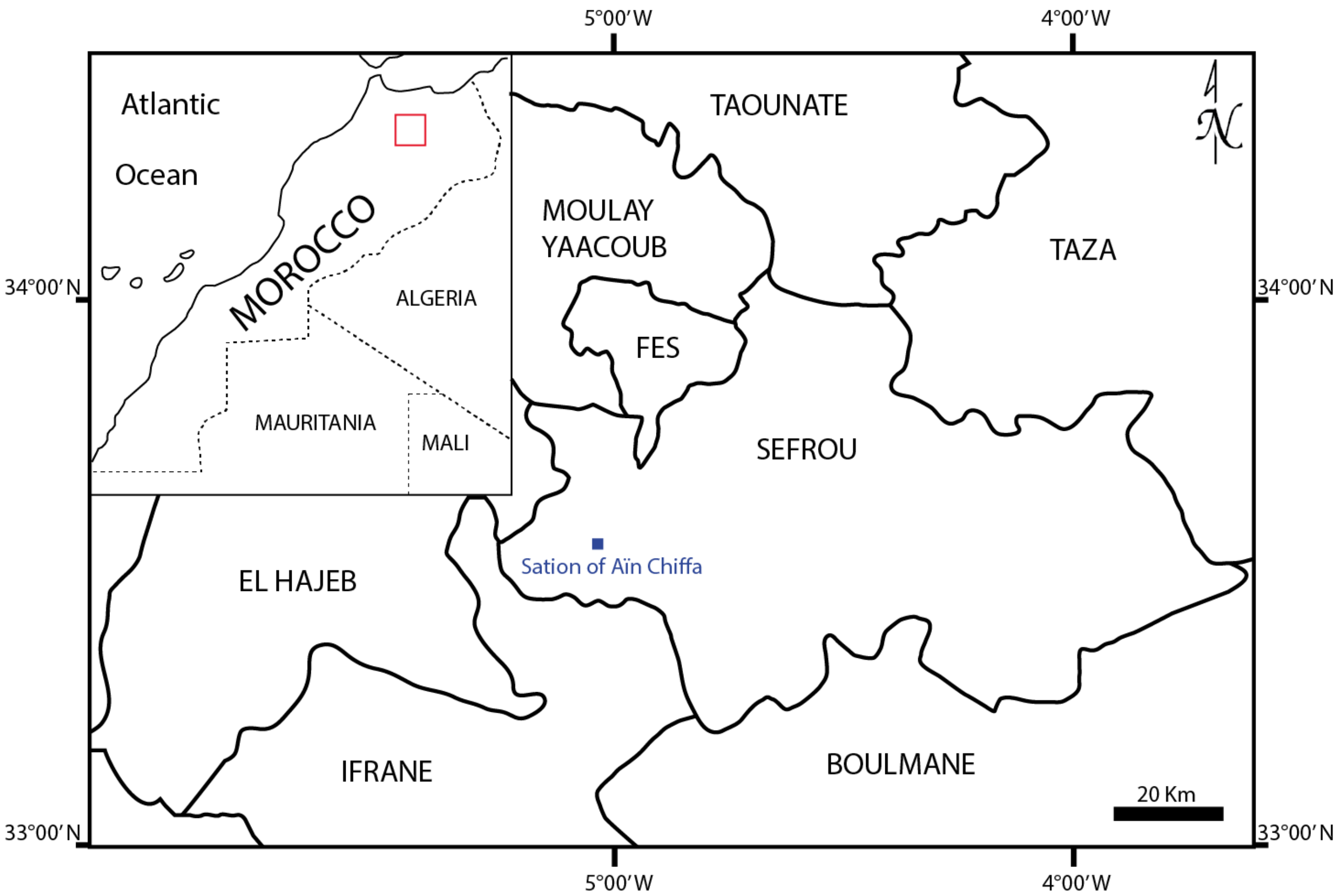

Figure 1. Geographical location of the study site.

RESULTS AND DISCUSSION

The present study is focused on phytoplankton biomass production in wastewater treatment station by natural lagooning at Aïn Chiffa, Sefrou-Morocco (Phytoplankton analysis). It was preceded by another study focusing on the efficiency of wastewater treatment by this station. This efficiency has been evaluated by studying a number of physical parameters (temperature, suspended matter (SM), electrical conductivity ... etc), chemical parameters (pH, dissolved oxygen, Nitrates, Ammonium and phosphorus...etc) and microbiological parameters (Rhenifel et *al.* 2020).

Phytoplankton analysis

Identification by optical microscope

Determination of the identity phytoplankton collected was carried out by obseration under the optical microscope the morpho-anatomic characters (shape, size, color) represented in the identification keys. 14 species of phytoplankton were inventoried during this study (Tab. 1). Frequency of each phytoplankton species changes from one sample to another.

Table 1. Groups of phytoplankton isolated and identidied from the treatment station of the wastewater by natural lagooning in Aïn Chiffa, Sefrou-Morocco.

Class	Order	Family	Genus	Species
Euglenophyceae	Euglenales	Euglenaceae	*Euglena*	*Euglena sp1*
				Euglena sp2
Chlorophyceae	Chlorococcales	Chlorococcaceae	*Chlorella*	*Chlorella sp*
			Follicularia	*Follicularia sp*
			Tetraedron	*Tetraedron minimum*
	Sphaeropleales	Selenastraceae	*Kirchneriella*	*Kirchneriellasp*
		Scenedesmaceae	*Scenedesmus*	*Scenedesmus quadricauda*
			Coelastrum	*Coelastrum sp*
	Chlamydomonadales	Chlamydomonadaceae	*Chlamydomonas*	*Chlamydomonas sp*
		Dunaliellaceae	*Dunaliella*	*Dunaliella sp*
Bacillariophyceae	Naviculales	Naviculaceae	*Navicula*	*Navicula sp*
	Bacillariales	Bacillariaceae	*Nitzschia*	*Nitzschia sp*
Cyanophyceae	Pseudanabaenales	Pseudanabaenaceae	*Pseudanabaena*	*Pseudanabaena catenata*
	Synechococcales	Merismopediaceae	*Synechocystis*	*Synechocystis sp*

Chlorella sp. is present with high densities in all water samples and throughout the study period. Also, this species is dominate and shows an important proliferation forming a bloom. *Chlamydomonas sp*. is present in small densities and only during the months of January, February and March. Another genus, characteristic of eutrophic waters, is present in the water samples analyzed throughout the study period: it is Euglena, represented by two species. Diatoms are less frequent and are represented by the genus Nitzschia and Navicula. Cyanobacteria are represented only by Pseudanabaena and Synechocystis. Some algal species such as *Euglena* and *Chlorella* are permanently present in wastewater treatment basins (Oudra 1990).

Phytoplankton genus listed in the anaerobic basins of the center of Aïn Chiffa are Euglenophyceae, Chlorophyceae, Bacillariophyceae and Cyanobacteria. These genus belong to the phytoplankton groups conventionally encountered in wastewater treatment stations by natural lagooning. Also, these genus are characterized by high biomass and low specific density, as for all phytoplankton genus detected in highly eutrophic environment.

Enumeration by revere microscopy

The technique of revere microscopy allows detailed recognition and quantification of micro-phytoplankton organisms (Utermöhl 1931). This technique allows the taxonomic analysis of phytoplankton cells and the calculation of the cellular biovolume or also of the phytoplankton biomass. This consists of evaluating the cell volume of each species and then report this volume to one or more geometric shapes calculated from the mean dimensions (Hillebrand 1999). The conversion factor of biovolumes into biomasses is: $106µm^3 = 1µg$.

Phytoplankton cells number in the lagoon basin varies between 20.10^{+6} cells/l and 243.10^{+6} cells/l, while at the station exit varies between 10.10^{+6} cells/l and 34.10^{+6} cells/l (Fig. 2). Highest number of phytoplankton cells is recorded in May 2016 (beginning of summer), characterized by a significant development of a bloom of *Chlorella* which can reach up to 84%.

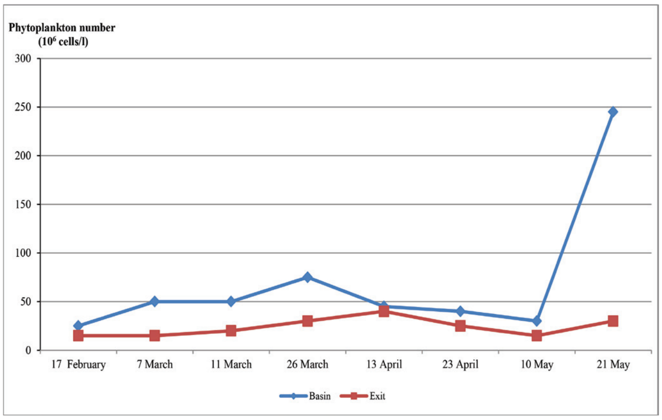

Figure 2. Spatio-temporal trends in the phytoplankton number in water samples collected from the two locations of the treatment station during the study period.

Results from studies cell biomass show that the treatment station by natural lagooning at Aïn Chiffa promotes the production of phytoplankton biomass rich in pollution-resistant species (*Chlorella sp* and *Euglena sp*) (Tab. 2).

Table 2. Specific biovolumes calculated for the most abundant algal species in the treatment station of Aïn Chiffa, Sefrou-Morocco.

Species	Cell volume (µm^3)
Chlorella sp	108
Euglena sp	1363
Chlamydomonas sp	294
Nitzischia sp	222
Navicula sp	252
Scenedesmus	1484

Chlorella sp.

Chlorella sp. is dominant in the Aïn Chiffa treatment station with high biomass concentrations. At the basin, biomass concentrations vary between 200 µg/l and 12 100 µg/l. At the exit of station, they vary between 20 µg/l and 2200 µg/l (Fig. 3).

Genus Chlorella is cited in other studies on the wastewater treatment. They verified the ability of *Chlorella sp*. in the laboratory to use the nutrients contained in the wastewater (Wang *et al*. 2010). The results obtained make it possible to conclude that these micro-algae can easily grow using the nutrients contained in the wastewater.

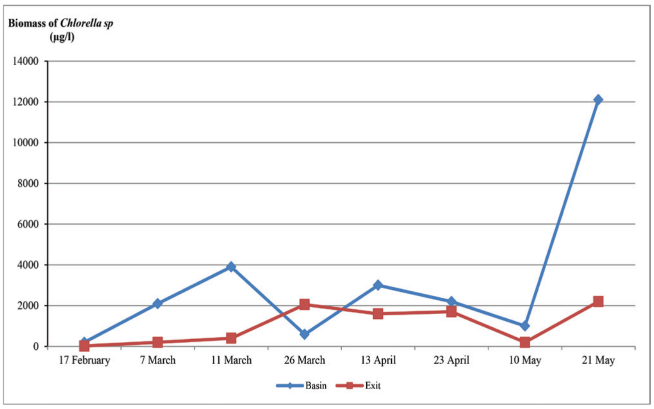

Figure 3. Spatio-temporal trends of the concentration of the biomass of *Chlorella sp* in water samples collected from the two locations of the treatment station during the study period.

Euglena sp.

Euglena sp. is known for its great tolerance to water polluted by trace metallic elements (Devars *et al*. 1998). It is the only group that represents Euglenophyceae. Bimonthly sampling results show that *Euglena sp*. biomass at the lagoon basin varies between 100 µg/l and 10 300 µg/l. However, at the exit of the station, they vary between 50 µg/l and 1900 µg/l (Fig. 4).

Changes in concentrations algal biomass recorded during the study period are mainly related to the interaction of several factors: (i) the Changes in climatic conditions (Temperature, Sunshine, Wind …etc.) and (ii) the nature of the effluent which feeds the lagoon basin with an organic and mineral charge favorable or harmful to algal development. Algal density decreases from the lagoon basin to the exit of the station.This decrease may be the result of the development of zooplankton populations.

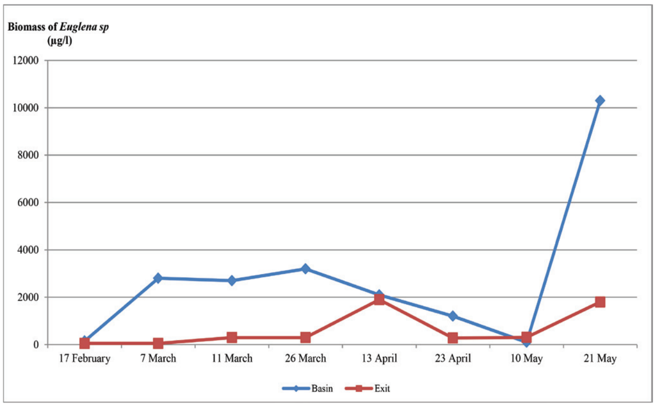

Figure 4. Spatio-temporal trends of the concentration of the biomass of *Euglena sp* in water samples collected from the two locations of the treatment station during the study period.

Chlorophyll (a) concentration by spectrophotometric

Phytoplankton can also be studied thanks to its photosynthetic pigments. The most commonly used method for estimating phytoplankton biomass from pigment content is to determine of the chlorophyll (a) concentration by spectrophotometry or fluorimetry (Aminot & Kérouel, 2004).

In the lagoon basin, chlorophyll (a) concentration varies between 140 µg/l and 2800 µg/l. At the exit of the station, they vary between 100 µg/l and 160 µg/l (Fig. 5). The highest chlorophyll concentrations (a) are detected in basin during the hot periods. This is associated with the development of the bloom of a *Chlorella sp*.

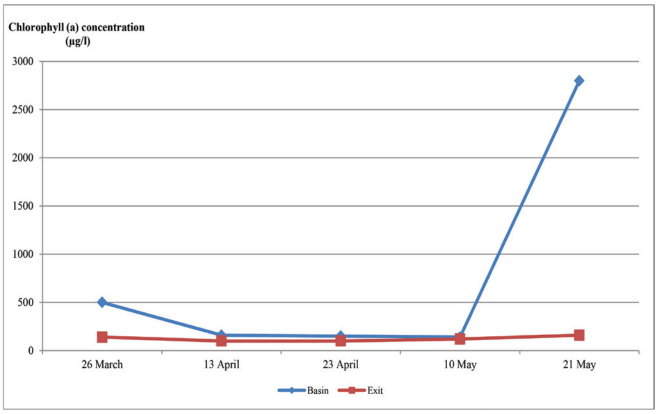

Figure 5. Spatio-temporal trends of chlorophyll (a) concentration in water samples collected from the two locations of the treatment station during the study period.

The figure 6 shows the relationship between phytoplankton number and the chlorophyll (a) concentration. The curves of chlorophyll (a) concentration and number of phytoplankton tend to be confounded. Chlorophyll (a) concentrations are often used as an index of algal productivity (Carlson 1977).

Variations in temperatures recorded in treatment station influence the development and the dominance of microalgae populations (Rhee & Gothman 1981). The pH in the lagoon basin is alkaline. This is due to development of micro-algae. The latter, by photosynthesis, assimilate dissolved CO2, which makes the environment alkaline (Edeline 1980 & Bambara 1985). Many studies have demonstrated the relationship between pH variations in wastewater treatment basins by natural lagooning and biological and biochemical activities of micro-algae, in particular the phenomenon of photosynthesis

(Davoust 1985; Chifaa 1987; Oudra 1990; Bouarab 2000; Fqih Berrada *et al*. 2000). Increase in the electrical conductivity in lagoon basin may be due to the increase in temperature and the strong mineralization (Bahlaoui 2000). Rhenifel *et al*. 2020 = BIS/SV/06-18 showed that when the station exit, average recorded value of electrical conductivity indicates that its water complies with the standard recommended for use in irrigation set at 12 m/cm (Joint decree from the Minister for Agriculture and Fisheries n°1276-01 of Octobre 17, 2002). Also, they showed that when the station exit, water is slightly cloudy compared to the entrance. Values obtained for turbidity at the outlet show that treated water is qualified as cloudy water (NM 03.7.010 (1989)).

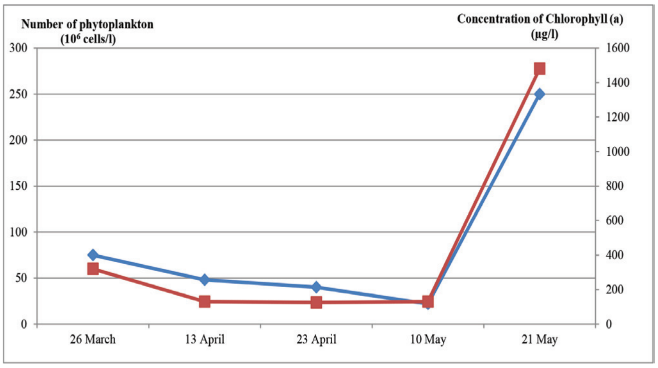

Figure 6. Temporal trends of chlorophyll (a) concentration and number of phytoplankton in water samples collected from of the treatment station during the study period.

Biochemical study by determination of carbohydrate, protein and lipid concentrations

Main cell constituents (proteins, carbohydrates and lipids) are considered as descriptors of phytoplankton biomass, indicators of physiological state and activity of algae.

Proteins.

From spatial point of view, phytoplankton proteins concentrations vary between 18 mg/l and 250 mg/l at the lagoon basin. However, on exit the station, they vary between 5 mg/l and 60 mg/l. From temporal viewpoint, phytoplankton proteins concentration increases with increasing phytoplankton density during the hot period (Fig. 7).

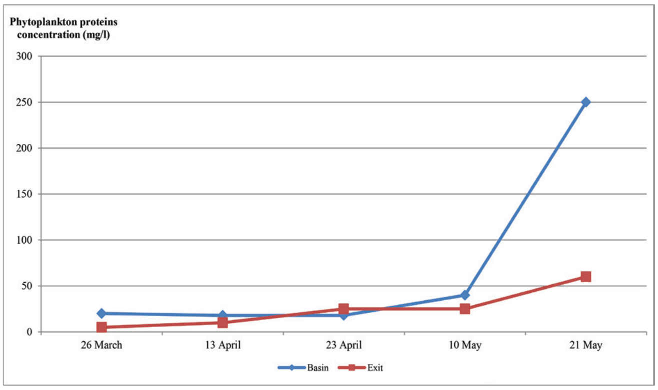

Figure 7. Spatio-temporal trends of phytoplankton proteins concentration in water samples collected from the two locations of the treatment station during the study period.

Carbohydrates.

From spatial point of view, at the lagoon basin, phytoplankton carbohydrates concentrations vary between 110 mg/l and 705 mg/l. On exit the station, they vary between 20 mg/l and 330 mg/l. From temporal viewpoint, the phytoplankton carbohydrates concentration increases with increasing phytoplankton density during the hot period (Fig. 8).

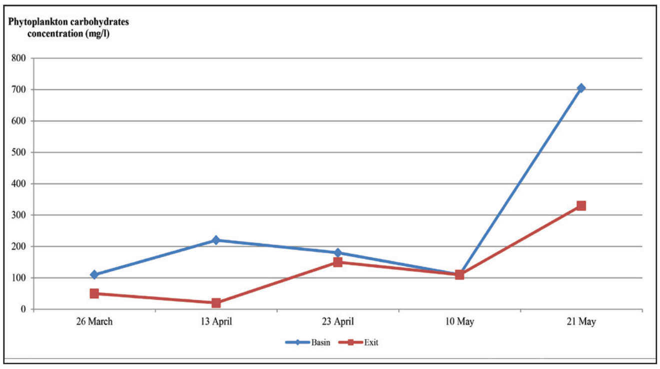

Figure 8. Spatio-temporal trends of phytoplankton carbohydrates concentration in water samples collected from the two locations of the treatment station during the study period.

Spatial evolution of phytoplankton protein and carbohydrate concentrations shows that concentrations are high in the lagoon basin than at the station exit. This is related to the algal development: more the temperature increases (January to May), more the proliferation of micro-algae is accentuated.

Lipids.

Phytoplankton lipids concentrations in the lagoon basin varies betwen range 15 mg/l and 67 mg/l. On exit the station, they vary between 2 mg/l and 18 mg/l (Fig. 9).

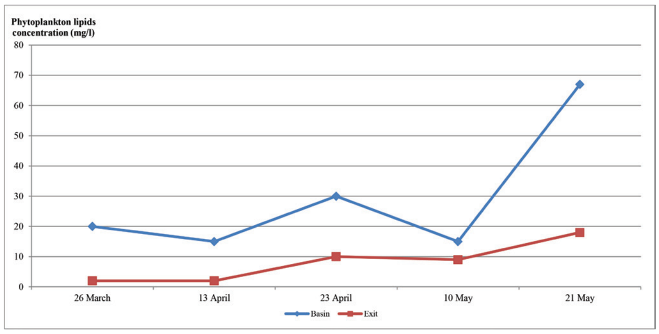

Figure 9. Spatio-temporal trends of phytoplancton lipids concentration in water samples collected from the two locations of the treatment station during the study period.

Protein/Carbohydrate ratio is considered as an index of physiological state of phytoplankton. For phytoplankton cell cultures this ratio is equal to 2. In our case and in basin, this ratio is less than 0.1. This shows that carbohydrates strongly dominate proteins, which may be due to algal metabolism which has a tendency to synthesize carbohydrates from raw organic matter. However, the nitrogen elements which are the determinants of the biosynthesis of the proteins, they undergo a strong ammonification and nitrification by the bacteria.

Quantity of proteins as well as that of lipids in phytoplankton biomass is strongly influenced by concentration of nitrogen and phosphorus compounds.

Nitrogenous compunds (ammonium and nitrate) come from oxidation of organic matter by bacteria of nitrogenous. Rhenifel *et al*. 2020 = BIS/SV/06-18 have similar results to those obtained by El Halouani (1995) and are much lower than those obtained by El Hamouri *et al*. (1987) and Bouhoum *et al*. (1995). The ammonium ion is preferentially absorbed when the algae have both the NH_4^+ and NO_3^- ions (DeBoer 1981). This explains the low concentrations of ammonium ion measured in summer, without neglecting the phenomenon of nitrification because the aquatic environment is well oxygenated. The concentrations of nitrate ion are very low in this station (less than 1 mg/l) (Rhenifel *et al*. 2020 = BIS/SV/06-18). These results agree with those obtained in other research works (Ratel *et al*. 1986, Bechac *et al*. 1987, El Halouani 1995 and Landreau 1987).

Phosphorus comes from domestic and agricultural activities. Its concentration in the station slightly exceeds the Moroccan quality standards for surface wastewater for the production of potable water (Order of the Minister of Equipment and the Minister in charge of spatial management, urban planning, housing and the environment (n° 1277-01of the 17 October 2002)).

CONCLUSION

Functioning of wastewater pretreatment station by natural lagooning at Aïn Chiffa in Sefrou city-Morocco varies during the study period (January-June 2016). There are two periods: (**i**) cold period (January-March) and (**ii**) hot period (April-June). First period is characterized by a low phytoplankton proliferation. Second period is characterized by an important of phytoplanktonic proliferation, which significantly alters the functioning of the station.

Phytoplanktonic populations play an essential role in the functioning of wastewater treatment station by natural lagooning. A variation of density and of composition of phytoplankton populations has a direct effect on the station's purifying performance. A detailed study of phytoplankton populations of station is therefore of major interest. Some phytoplankton species are characterized by their almost permanent presence in the basins of lagooning (Chlorophyceae and Euglenophyceae). They generally belong to groups known for their adaptation to difficult living conditions and typical of eutrophic water locations. Distribution of these species and their abundance are determined by their physiological capacity to adapt to changing physico-chemical conditions of the environment (Hee-Mock & Rhee 1991). Succession of phytoplankton species is often explained by differences in their specific eco-physiological constants (Guerri *et al*. 1981). Quantitative study of phytoplankton populations is carried by determination of chlorophyll (a) concentration and cell enumeration. Estimation of the phytoplankton biomass is completed by determination of concentration of cellular constituents (carbohydrates, proteins and lipids). Spatio-temporal variations in protein concentrations are similar to those of lipids and not to those of carbohydrates. Algal biomass is controlled in a lagooning station by several factors, such as light intensity, photoperiod, temperature, pH, nutrients (Phosphorus, nitrogen…etc.), CO2 and O2 concentrations, and physiological state of cells (Bouarab *et al*. 2002).

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